REMARKS

The present application is directed to methods, kits and disposable units for performing a nucleic acid amplification reaction. Claims 1-6 and 8-35 were pending prior to the issuance of the July 31, 2006, Office Action. Following entry of this amendment, Claims 1, 3-6 and 8-35 will be pending. Claims 1, 3, 17, 27 and 29 are currently amended. Claims 2 and 7 are cancelled without prejudice. No new matter is added, and support for the amendments may be found throughout the specification and in the original claims.

Formality

Applicants respectfully request entry of Amendment to the Specification as provided herein. The Examiner stated on page 2 of the Office Action mailed July 31, 2006, that the response received September 6, 2005 was held non-responsive due to formality and that applicants were required to re-enter the Amendment to the Specification. Applicants submit no new matter is added by the amendment.

Claim rejections under 35 U.S.C. §103 (a)

In the Non-Final Office Action mailed July 31, 2006, the Examiner rejected Claims 1-5, 8-18, 21, 25-29, 32 and 33 under 35 U.S.C. §103(a), as being unpatentable over Beutler *et al.* (U.S. Patent No. 5,234,811) in view of Ronchi (U.S. Patent No. 6,372,484) as evidenced by Heritz *et al. (Journal of Urology*, 1997*)*. Applicants respectfully submit that the amendments to the claims overcome the rejection.

Claims 1 and 29 have been amended to specify that the reaction mixture contains a buffer system having a pH **above pH 8.3**. The present application teaches that addition of a **high pH buffer (above pH 8.3)** in an amplification reaction allows for use of amplification reaction vessels composed of a **wide range of materials**, without adverse consequences.

The applicants of the present application have identified a particular problem, namely that reaction vessels made of certain materials will inhibit nucleic acid amplification reactions. Applicants developed an innovative solution to this problem. They discovered that the addition of a high pH buffer to the amplification reaction overcomes amplification inhibition.

Although the cited prior art acknowledges that incompatibility of a reaction vessel with an amplification mixture can lead to inhibition of an amplification reaction, none of the prior art references contemplate use of a high pH buffer in the reaction mixture. Instead, the prior art references attempt to solve the amplification inhibition problem by either changing the material of the reaction vessel to a more inert form (as taught by Ronchi) or by coating the material with a non-reactive material (as taught by Wilding). Applicants unexpectedly discovered that incompatibility problems could be overcome by adjusting the pH to be above **pH 8.3**. Applicants respectfully submit that this beneficial and advantageous solution is not taught or suggested by the prior art.

Beutler *et al.* discloses an amplification reaction that uses Tris-HCl.

Ronchi discloses an apparatus that is capable of carrying out amplification reactions and capillary electrophoresis but is silent with respect to the pH at which amplification should be carried out.

The Examiner concluded, on page 11 of the Office Action, that a claim drawn to a method involving a device, which is known, and a PCR condition, which is also known, could only be patentable when the combination of the device and the specific reaction condition produced **effects which merit secondary consideration** (i.e. unexpected results). As discussed above, applicants respectfully submit the claimed method produces an **unexpected** result. Specifically, the choice of materials suitable for making the disposable unit is wider than when amplification is carried out in a buffer condition of pH 8.3 or below. This unexpected result is not taught or suggested by the cited references.

In particular, Table 4 of the instant application discloses that when a buffer of pH 8.3 is used, a blocking agent, bovine serum albumin (BSA), is **required** to avoid inhibition of the amplification reaction. In contrast, Table 3 of the present application teaches that when a buffer having a pH **above 8.3** is used, successful amplifications can be carried out in vessels composed of a wide variety of materials in the **absence** of BSA. For example, successful amplification occurs in reaction vessels with polycarbonate, polystyrene and/or glass surfaces, without the need for BSA. Therefore, the benefit of the present method is to widen the choice of amplification reaction vessel materials without concern that the materials will inhibit the amplification reaction.

The Examiner concluded that it would be obvious to combine the amplification conditions of Beutler *et al.* with the apparatus of Ronchi because Ronchi discloses a closed environment that precludes loss of volume and possibility of contamination of PCR products via vaporization of the sample fluid during the PCR cycle and this is desirable. Applicants wish to highlight that, although Beutler *et al.* mention one example where PCR is carried out at pH 8.8, Beutler *et al.* also teaches that amplifications can be carried out **at pH 8.3** (Beutler *et al.*, column 10, line 30), and more preferably at about **pH 8** (column 10, line 8). It is submitted that Beutler *et al.* therefore adds nothing to the general art other than that amplification reactions can be carried out at a variety of pHs both above and below 8.3. Applicants submit that one of ordinary skill reading Beutler *et al.* in combination with Ronchi would therefore **not** be motivated to select a pH buffer condition in **excess** of 8.3. Based on the preferred teachings of Beutler *et al.*, one could argue that Beutler *et al.* **teach away** from the present method because Beutler *et al.* disclose an exemplary amplification buffer **at** pH 8.3 (column 10, lines 29-30) whereas the claimed method requires a buffer system having a pH **above** 8.3.

Ronchi identifies a potential compatibility problem between the vessel and amplification reaction mixture (Ronchi, column 8, lines 43-45), but Ronchi's solution to the problem is to **change the material** from which the reaction vessel is made. In the example, Ronchi specifies that Scotch™ tape (Scotch™ #850) should be used in order to circumvent the incompatibility problem. Combining Ronchi with Beutler *et al.* might therefore lead one of ordinary skill in the art to carry out an amplification reaction in a reaction vessel made of Scotch tape. Ronchi and Beutler *et al.*, therefore, fail to teach or suggest the claimed method. Applicants respectfully submit that neither reference teaches or suggests that one of ordinary skill in the art arrive at the subject matter claimed herein. The mere fact that Ronchi *et al.* and Beutler *et al.* can be combined does not render the claimed method obvious for at least the foregoing reasons.

Heritz *et al.* discloses an amplification reaction carried out using a Tris buffer of pH 8.5 at 25°C. However, the main topic of the Heritz paper is not amplification reaction conditions, but rather interstitial cystitis and the role of non-culturable microorganisms in disease (see title and objective of paper). No mention is made by Heritz *et al.* of potential incompatibility problems between reaction vessels and amplification reactions. Accordingly, applicants respectfully submit that Heritz adds only that the pH of the Tris buffer used in the

amplification reaction should be measured at 25°C. Applicants respectfully submit that this information fails to motivate one of ordinary skill in the art to derive the claimed method. The combination of Beutler *et al.* Ronchi and Heritz *et al.* therefore fails to make the claimed method obvious.

With regard to independent Claims 27 and 29, Beutler *et al.,* Ronchi and Heritz *et al.* fail to teach or suggest the combination of a disposable unit for conducting a thermal cycling reaction or a polymerase chain reaction with a buffer system comprising a buffer having a pH in excess of 8.3, wherein the layers of the disposable unit comprising a thermally conducting layer and a facing layer are **adhered together by means of a biocompatible adhesive**.

Applicants respectfully submit that independent Claims 1, 27 and 29 are non-obvious over the prior art of record for at least the foregoing reasons. In addition, Claims 3-5, 8-18, 21, 25-26, 28, 32 and 33 depend directly or indirectly from amended Claim 1. As discussed above, applicants respectfully submit that the pending claims are non-obvious over the teachings of Beutler *et al.,* Ronchi and Heritz *et al.* Accordingly, applicants respectfully request withdrawal of the Examiner's rejection of Claims 1-5, 8-18, 21, 25-29, 32 and 33 under 35 U.S.C. §103(a).

The Examiner rejected Claim 6 under 35 U.S.C. § 103(a) as being unpatentable over Beutler *et al.* (already of record) in view of Ronchi (already of record), as evidenced by Heritz *et al.* (already of record) as applied to Claims 1-5, 8-18, 21, 25-29, 32 and 33 above and further in view of Moss *et al.* (US Patent 5,386,021). Applicants respectfully submit that the amendments to the claims overcome the rejection.

Applicants reiterate their remarks in response to Beutler *et al.* and Ronchi *et al.* as evidence by Heritz *et al.* as applied to Claims 1, 3-5, 8-18, 21, 25-29, 32 and 33 above. Additionally, applicants respectfully submit that the deficiencies of Beutler *et al.*, Ronchi *et al.* and Heritz *et al.* are not satisfied by Moss *et al.* for at least the following reasons.

Moss *et al.* disclose an amplification reaction carried out in the presence of 0.1% Tween (a detergent)(column 12, lines 16-19). Moss' disclosure falls outside the scope of amended Claim 1 because it fails to satisfy the **pH requirement**. Instead, Moss *et al.* use a Tris-

HCl solution at pH 8.3. Combining Moss with the other cited references might therefore lead one of ordinary skill in the art to carry out an amplification reaction at pH 8.3 in the presence of 0.1% detergent. Accordingly, Moss *et al.* fails to suggest or disclose a method of carrying out an amplification reaction in a disposable unit, wherein the reaction mixture contains a buffer system having a pH **above** 8.3 as recited in amended Claim 1. Claim 6 depends directly from amended Claim 1.

For at least the foregoing, applicants respectfully submit that Claim 6 is non-obvious over the prior art. Accordingly, applicants respectfully request withdrawal of the Examiner's rejection of Claim 6 under 35 U.S.C. §103(a).

The Examiner rejected Claims 19, 20, 30 and 31 under 35 U.S.C. § 103(a) as being unpatentable over Beutler *et al.* in view of Ronchi, as evidenced by Heritz *et al.* as applied to Claims 1-4, 8-18, 21, 25-29, 32 and 33 above, and further in view of Little *et al.* (US Patent 6,077,669). Applicants respectfully submit that the amendments to the claims overcome the rejection..

Applicants reiterate their remarks in response to Beutler *et al.* and Ronchi as evidenced by Heritz *et al.* as applied to Claims 1, 3-4, 8-18, 21, 25-29, 32 and 33 above. Additionally, applicants respectfully submit that the deficiencies of Beutler *et al.*, Ronchi and Heritz *et al.* are not satisfied by Little *et al.* for at least the following reasons. Little *et al.* fail to suggest or disclose a method of carrying out an amplification reaction in a disposable unit, wherein the reaction mixture includes a buffer system having a **pH above 8.3** as recited in amended Claim 1.

Little *et al.* disclose dried amplification reagents in combination with nucleic acid probes (column 2, lines 30-34). Applicants respectfully submit that the paragraph of Little cited by the Examiner **teaches away** from the use of dried reagents because the same paragraph mentions that a method using those dried reagents caused "an unreproducible fluorescence detection signal". The Examiner concluded that the unreproducible fluorescence detection signal arose from the pre-dried nucleic acid probes rather than the pre-dried amplification reagents. Applicants respectfully submit that the Examiner cannot make such a conclusion based on the disclosure of Little *et al.* Applicants agree that the cause of an unreproducible detection signal

could be due to the fact that there is something wrong with the dried nucleic acid probe. However, another possible cause for the unreproducible detection signal is that something was wrong with the amplification as a result of the dried amplification reagents. In other words, if there is no signal or no reproducible signal from a reaction, it is not possible to tell whether the amplification reaction is incomplete/ineffective or whether it is the signaling means (pre-dried nucleic acid probes) that is at fault. Applicants submit it is not apparent from the passage of Little *et al.* that successful amplification can be carried out using dried reagents and in fact, applicants respectfully submit that Little *et al.* teach away from using dried reagents for amplification because the result achieved is unreliable. Accordingly, applicants respectfully submit that one of ordinary skill in the art would not be motivated to modify the teachings of Little *et al.* to arrive at the subject matter of Claims 19, 20, 30 and 31.

For at least the foregoing, applicants respectfully submit that Claims 19, 20, 30 and 31 are non-obvious over the prior art. Accordingly, applicants respectfully request withdrawal of the Examiner's rejection under 35 U.S.C. §103(a).

The Examiner has rejected Claims 22-24 under 35 U.S.C. § 103(a) as being unpatentable over Beutler *et al.* in view of Ronchi, as evidenced by Heritz *et al.* as applied to Claims 1-4, 8-18, 21, 25-29, 32 and 33 above, and further in view of Danssaert *et al.* (US Patent 5,525,300). Applicants respectfully submit that the amendments to the claims overcome the rejection.

Applicants reiterate their remarks in response to Beutler *et al.* and Ronchi *et al.* as evidence by Heritz *et al.* as applied to Claims 1-4, 8-18, 21, 25-29, 32 and 33 above. Additionally, applicants respectfully submit that the deficiencies of Beutler *et al.*, Ronchi *et al.* and Heritz *et al.* are not satisfied by Danssaert *et al.* for at least the following reasons. Danssaert *et al.* fails to suggest or disclose a method of carrying out an amplification reaction in a disposable unit, wherein the disposable unit comprises a reaction mixture containing a buffer system having a **pH above 8.3** as recited in amended Claim 1. Claims 22-24 depend directly or indirectly on amended Claim 1. Accordingly, applicants respectfully submit that one of ordinary skill in the art would not be motivated to modify the teachings of Danssaert *et al.* to arrive at the subject matter of Claims 22-24.

The Examiner alleges Danssaert discloses an apparatus that falls within the apparatus as described in Claims 22 to 24 and that it would be obvious to combine Danssaert with the other cited art because of the advantage of determining the optical temperatures required in an amplification reaction. Applicants have interpreted the Examiner's statement to mean determining "optimum" temperatures. If this was not as intended, applicants kindly request clarification.

Applicants respectfully submit Danssaert *et al.* relates to a temperature cycling machine for carrying out nucleic acid amplification, DNA sequencing and the like (Danssaert column 1, lines 5-10). There is no suggestion in Danssaert *et al.* that the type of material used for the disposable unit (*i.e.* vessel) in which the amplification reaction is carried out may be important. This is unsurprising given that the patent is directed to a machine for carrying out amplification rather than the amplification reaction itself or what the amplification reaction can be carried out in. There is a suggestion in Danssaert *et al.* that the gradient block of the machine can be made of brass (column 4, lines 27-28) and that the amplification reactions could be placed in the gradient block directly (column 3, lines 28-33). However, any possible incompatibility problem between the amplification reaction mixture and the material making up the gradient block is not contemplated by Danssaert *et al.* Applicants submit Danssaert *et al.* fails to motivate one of ordinary skill in the art to arrive at the claimed method because Danssaert *et al.* remains silent with respect to the problem at hand. Applicants therefore conclude that Danssaert *et al.* fails to motivate one of ordinary skill to use a high pH buffer to overcome incompatibilities between amplification materials and the amplification reaction.

For at least the foregoing, applicants respectfully submit that Claims 22-24 are non-obvious over the cited prior art. Accordingly, applicants respectfully request withdrawal of the Examiner's rejection under 35 U.S.C. §103(a).

Claim rejections under 35 U.S.C. §102 (b)

In the Non-Final Office Action mailed July 31, 2006, the Examiner rejected Claims 1, 2, 8-12, 14, 15, 16, 18, 21, 22, 25-29, 32 and 33 under 35 U.S.C. §102(b), as being

anticipated by Wilding *et al.* (US Patent 5,587,128). Applicants respectfully submit that the amendments to the claims overcome the rejection.

Applicants respectfully submit that the claimed method requires use of a **high pH buffer (above pH 8.3)** in an amplification reaction allowing for a wider range of materials to be used to make the disposable unit in which the amplification reaction is carried out. Applicants unexpectedly discovered that use of a buffer having a pH above 8.3 can overcome incompatibility problems. Applicants respectfully submit that this limitation, as recited in amended Claim 1, is not taught or suggested by Wilding *et al.*

Wilding *et al.* disclose the use of a Tris buffer having a pH of 8.6. However, the buffer is **removed** from the amplification reaction chamber by applying a negative pressure to the exit port of the device **before** the amplification reaction is carried out (column 27, lines 39-40). The Tris buffer is therefore used in a process for **pre-coating** the amplification reaction chamber. Wilding *et al.* does **not** disclose a method of carrying out an **amplification reaction** with a buffer system having a pH in excess of 8.3. Therefore, Claims 1, 2, 8-12, 14, 15, 16, 18, 21, 22, 25 and 26 are not anticipated by Wilding *et al.*

Furthermore, applicants submit that Wilding *et al.* fail to anticipate the amended claims because, although Wilding *et al.* acknowledges the incompatibility problem (column 5, lines 27-29), the solution devised by Wilding *et al.* is to **coat the reaction vessels with a silane** (column 5, line 52). Therefore, Wilding *et al.* **teach away** from the claimed method for two reasons. First, Wilding *et al.* teach away because they provides an **alternative solution** to the problem solved by the present application (i.e. coat the reaction vessels with silane). Second, Wilding *et al.* teach away because they teach that a high pH buffer needs to be **removed** from a vessel before an amplification reaction is carried out (column 27, lines 39-44), implying that a high pH buffer may be **detrimental** to the amplification reaction. Accordingly, applicants respectfully submit Wilding *et al.* fail to anticipate Claims 1, 2, 8-12, 14, 15, 16, 18, 21, 22, 25 and 26.

With respect to Claims 27 and 29, the claims have been amended to specify that the layers are **adhered together** by means of a **biocompatible adhesive**. Wilding *et al.* fails to disclose this feature and cannot therefore anticipate these claims. Accordingly, applicants

respectfully request withdrawal of the Examiner's rejection of Claims 1, 2, 8-12, 14, 15, 16, 18, 21, 22, 25-29, 32 and 33 under 35 U.S.C. §102(b).

Claim rejections under 35 U.S.C. §103 (a)

In the Non-Final Office Action mailed July 31, 2006, the Examiner rejected Claims 3-5 under 35 U.S.C. §103(a), as being unpatentable over Wilding *et al.* in view of Beutler as evidenced by Heritz *et al.*. Applicants respectfully submit that the amendments to the claims overcome the rejection.

Applicants reiterate their remarks in response to Beutler *et al.* and Heritz *et al.* as discussed above. Additionally, applicants respectfully submit that the deficiencies of Beutler *et al.* and Heritz *et al.* are not satisfied by Wilding *et al.* for at least the following reasons.

As already explained above, Wilding *et al.* teaches coating a reaction vessel in order to overcome inhibitory effects of an amplification reaction by the material making up the vessel. Wilding *et al.* fails to suggest or disclose that a high pH buffer could solve the problem of incompatibility. In fact, high pH buffers are removed from the reaction vessel of Wilding **before** amplification is performed.

Applicants respectfully submit that if one were to combine Wilding *et al.* and Beutler *et al.* one might carry out an amplification reaction in a coated vessel where the pH of the reaction is preferably about 8 or 8.3. Additionally, since Wilding *et al.* teach that high pH buffers are to be avoided in the amplification mixture, a skilled person combining the two cited references is unlikely to use a high pH buffer.

The contribution Heritz *et al.* brings is that the pH of a Tris buffer (in this case, a buffer having a pH of 8.5) is measured at 25°C and that this buffer is suitable for a particular amplification reaction. Since Beutler *et al.* already disclose a range of pHs (pH 7-9, see Beutler, column 10, line 7) for amplification, which covers pH 8.5 (in that pH 8.5 falls within the range of pH 7-9), the most Heritz *et al.* contributes is that the pH should be measured at 25°C. Beutler *et al.* then teaches that a preferred pH of 8 and 8.3 should be used. Combining the two documents would lead a skilled person to use a buffer of 8 or 8.3 wherein the pH was muasured at 25°C. Applicants respectfully submit, therefore, that Heritz *et al.* fails to address the deficiency in the

obviousness argument arising from combining Wilding *et al.* and Beutler *et al.* alone, and thus adding Heritz *et al.* does not motivate one of ordinary skill in the art to derive the claimed method. Accordingly, applicants respectfully request withdrawal of the Examiner's rejection of Claims 3-5 under 35 U.S.C. §103(a).

The Examiner rejected Claim 6 under 35 U.S.C. § 103(a) as being unpatentable over Wilding *et al.* in view of Moss *et al.* Applicants respectfully submit that the amendments to the claims overcome the rejection.

Applicants reiterate their remarks in response to Wilding *et al.* and Moss *et al.* as described above.

Moss *et al.* disclose an amplification reaction carried out in the presence of 0.1% Tween (a detergent)(column 12, lines 16-19). Moss' disclosure falls outside the scope of amended Claim 1 because it does not satisfy the **pH requirement**. Instead, Moss *et al.* use a Tris-HCl solution at pH 8.3. Combining Moss *et al.* with Wilding *et al.* might lead one of ordinary skill in the art to carry out an amplification reaction at pH 8.3 in the presence of 0.1% detergent wherein the reaction vessel is coated. Accordingly, Moss *et al.* and Wilding *et al.* fail to suggest or disclose a method of carrying out an amplification reaction in a disposable unit, wherein the reaction mixture includes a buffer system wherein **the pH is above 8.3**, as recited in amended Claim 1. Claim 6 depends directly from amended Claim 1.

For at least the foregoing, applicants respectfully submit that Claim 6 is non-obvious over the cited references. Accordingly, applicants respectfully request withdrawal of the Examiner's rejection of Claim 6 under 35 U.S.C. §103(a).

The Examiner rejected Claims 19, 20, 30 and 31 under 35 U.S.C. § 103(a) as being unpatentable over Wilding *et al.* in view of Corless *et al.* (WO 98/09728). Applicants respectfully submit that the amendments to the claims overcome the rejection..

Applicants reiterate their remarks in response to Wilding *et al.* discussed above. Additionally, applicants respectfully submit that the deficiencies of Wilding *et al.* are not satisfied by Corless *et al.* for at least the following reasons. Corless *et al.* fails to suggest or disclose a method of carrying out an amplification reaction in a disposable unit wherein the

reaction mixture comprises a buffer system having a **pH above 8.3** as recited in amended Claim 1.

With respect to Claim 30, neither Wilding *et al.* nor Corless *et al.* disclose a unit wherein the **layers are adhered together by means of a biocompatible adhesive**. Thus, the claimed method cannot be made obvious in view of Wilding and Corless.

For at least the foregoing, applicants respectfully submit that Claims 19, 20, 30 and 31 are non-obvious over the cited prior art. Accordingly, applicants respectfully request withdrawal of the Examiner's rejection under 35 U.S.C. §103(a).

The Examiner also rejected Claims 13 and 16 under 35 U.S.C. § 103(a) as being unpatentable over Wilding *et al.* in view of Dubrow *et al.* (US Patent 6,488,897). Applicants respectfully submit that the amendments to the claims overcome the rejection..

Applicants reiterate their remarks in response to Wilding *et al.* discussed above. Additionally, applicants respectfully submit that the deficiencies of Wilding *et al.* are not satisfied by Dubrow *et al.* for at least the following reasons. Dubrow *et al.* fails to suggest or disclose a method of carrying out an amplification reaction in a disposable unit wherein the reaction mixture comprises a buffer system wherein the **pH is above 8.3** as recited in amended Claim 1.

For at least the foregoing, applicants respectfully submit that Claims 13 and 16 are non-obvious over the cited prior art. Accordingly, applicants respectfully request withdrawal of the Examiner's rejection under 35 U.S.C. §103(a).

The Examiner rejected Claims 22-24 under 35 U.S.C. § 103(a) as being unpatentable over Wilding *et al.* in view of Danssaert *et al.*. Applicants respectfully submit that the amendments to the claims overcome the rejection..

Applicants reiterate their remarks in response to Wilding *et al.* and Danssaert *et al.* already discussed in detail above. Briefly, Danssaert *et al.* relates to a temperature cycling machine and Wilding *et al.* relates to coating reaction vessels to overcome inhibitory effects of the vessel material on amplification reactions. Applicants respectfully submit that combining the two references might lead one of ordinary skill in the art to coat an amplification reaction vessel

with a suitable coating to prevent incompatibility and then to carry out the amplification reaction in that vessel using the machine disclosed in Danssaert *et al.* Applicants respectfully submit neither document discloses that a buffer **above pH 8.3** could be used in the amplification reaction as recited in amended Claim 1. Additionally, applicants submit that neither reference teaches that such a buffer could solve incompatibility problems.

For at least the foregoing, applicants respectfully submit that Claims 22-24 are non-obvious over the cited prior art. Accordingly, applicants respectfully request withdrawal of the Examiner's rejection under 35 U.S.C. §103(a).

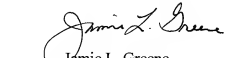
CONCLUSION

The foregoing is submitted as a complete Response to the Non-Final Office Action mailed on July 31, 2006. For at least the reasons given above, applicants submit that the claims in the present application are in condition for allowance, and such action is courteously solicited.

No additional fees are believed due; however, the Commissioner is hereby authorized to charge any deficiency, or credit any overpayment, to Deposit Account No. 11-0855.

If the Examiner believes that any informalities remain in the case, which may be corrected by Examiner's amendment, or that there are any other issues which can be resolved by a telephone interview, a telephone call to the undersigned attorney at (404) 815-6500 is respectfully solicited.

Respectfully submitted,



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